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Carcinogenesis of Mammary Gland in Rat*

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I. Introduction

There exists a considerable amount of information concerning experimental studies of mammary tumors in mice. The influence of endocrine, viral, and genetic factors on the etiology, pathogenesis, and control of mammary neoplasia in mice has been extensively investigated by workers in various scientific disciplines. The ease with which various inbred strains of mice can be maintained and propagated in the laboratory and the classical conception of the genetic factors involved in the origin of mammary cancer are important reasons why, for decades, investigators have had little inclination to put any concentrated effort into the study of mammary neoplasia in rats, even though this is an area that seems worthy of serious attention.

Some of the reasons which lend special interest to the study of rat mammary tumors are the following: 1. Malignant mammary tumors can be induced easily at young ages, thus making it possible to study the factors influencing the growth rate of tumors, the production of metastases, and the control of tumor growth through the animals' life span. 2. Induced rat tumors are curiously sensitive to hormonal manipulations, and in this respect resemble human mammary cancer. 3. No viral factors have so far been unequivocally demonstrated.

Although both benign and malignant mammary rat tumors occur spontaneously, the incidence of adenocarcinomas is low, and cancer often develops late in life. Spontaneous mammary tumors in rats are thus not suitable for experimental research. BULLOCK AND CURTIS (1930) reported the observation of only two adenocarcinomas in 94 spontaneous breast tumors in 489 rats. In a study of 15,625 rats, DUNNING AND CURTIS (1956) noted only two malignant tumors among 32 spontaneous mammary tumors. Perhaps the highest incidence of spontaneous mammary adenocarcinoma in rats has been observed in the Sprague-Dawley random-bred strain. DAVIS *et al.* (1956) reported a 10% incidence of adenocarcinoma in 150 female rats of this strain during a life-span of 760 days. THOMPSON *et al.* (1961) observed only one adenocarcinoma among the spontaneous mammary tumors in 52 of 125 Sprague-Dawley rats. In the present author's own laboratory, 50 spontaneous mammary adenocarcinomas occurred among 1000 rats observed for an average of 540 days.

Obviously, an induced mammary cancer would be most desirable for experimentation if it could meet the following criteria: 1. uncomplicated induction technique; 2. short induction period; 3. nearly 100% tumor incidence, and 4. production of hormone-dependent tumors. Rat mammary tumor can be induced either by hormones or chemical carcinogens. The present chapter is focused on work done in the field of experimental mammary tumorigenesis by means of chemical carcinogens, especially work concerned with the mechanism of carcinogenesis in the mammary gland. Particular attention will be given to work done in the author's own laboratory. Currently a need exists for selective review of certain basic questions concerning mammary carcinogenesis, and the author has attempted to fulfill that need, rather than to provide complete coverage of the literature.

II. Effects of Pituitary and Steroid Hormones

1. Mammary tumor induction by anterior pituitary hormones

The best-known influences on normal cellular proliferation are exerted by the pituitary hormones and the gonadal and adrenocortical steroid hormones. Besides ovarian hormones, the anterior pituitary hormones are known to be of importance in the normal development of the mammary gland. Stimulation of the mammary gland by anterior pituitary hormone and the development of mammary tumors in the stimulated mammary gland have been investigated. Earlier, Evans and Simpson (1931) injected an alkaline aqueous extract of the anterior pituitary into Long-Evans strain rats, and observed the induction of mammary hyperplasia. The same anterior pituitary extract had no such effect if the rats were ovariectomized. These authors noted the development of fibroadenoma in 10 of 16 female rats receiving daily injections of the anterior pituitary extract for as long as 11 months. Male rats similarly treated did not develop any tumors. When highly purified pituitary growth hormone became available, Moon *et al.* (1950) repeated the experiment in Long-Evans strain rats, and obtained fibroadenomas in 7 of 15 female rats receiving this purified hormone over periods up to 485 days. In the control group, 3 out of 15 rats developed multiple fibroadenomas. The growth hormone apparently increased the frequency of mammary fibroadenoma, but did not shorten the latent period of tumor development. In a similar ex-

periment, growth hormone was administered to hypophysectomized rats for a similar time period, but no tumors were observed (Moon *et al.*, 1951). Administration of purified growth hormone alone to hypophysectomized rats also failed to induce mammary gland growth and hyperplasia. No mammary cancer has ever been observed in rats repeatedly injected with growth hormone. The results of these experiments suggest that growth hormone has little, if any, effect on the induction of mammary tumors, at least in this rat strain.

It is interesting to note that although relatively purified lactogenic hormone (Prolactin) has been available in recent years, its effect on mammary tumors induction in rats has not been reported. Mühlbock and Boot (1959) showed that increased stimulation of the mammary gland by subcutaneously grafted hypophyses, which produce only prolactin, induces mammary tumors in mice without the milk factor. However, these investigators failed to provide conclusive evidence that the induction of mammary tumors in these mice was due to the effect of prolactin, since the mice used in these experiments had not been ovariectomized. It is now well established that prolactin can perform mammotrophic, lactogenic, and luteotrophic functions (Dao, 1962). Mühlbock and Boot (1959) found that the prolactin released by the pituitary isograft, which possessed luteotrophic activity, apparently stimulated and maintained the *corpora lutea* of the ovaries, thereby producing a continued state of pseudopregnancy. Earlier that same year, Dao and Sunderland (1959) had reported that both pregnancy and pseudopregnancy enhanced the induction of mammary cancer by chemical carcinogens.

Perhaps the most interesting studies concerning the 'mammotrophic' hormones of the pituitary were the investigations of Furth *et al.* (1956), who showed estrogen-induced pituitary tumors to be both mammotrophic and somatotrophic. When transplanted into an estrogen-treated recipient, such a tumor grew, markedly stimulating both the growth and the secretory activity of the mammary gland. There has been no evidence at all, however, that 'mammotrophic' hormones of the pituitary can induce mammary cancer. In rats, subcutaneously transplanted hypophyses secrete prolactin and induce marked growth of the mammary glands, but the present authors has never observed a mammary tumor in rats which had received such implants (Dao, 1962; Dao and Gawlak, 1963).

2. Mammary tumor induction by prolonged administration of estrogens

Various experiments with mice have provided overwhelming evidence that the ovaries play a major role in the etiology of mammary cancer, primarily because of the estrogenic substances which they secrete.

Estrogen-induced mammary cancer in rats is of special significance and interest, since spontaneous mammary cancer in rats is rare (under ordinary conditions), and the continuous administration of estrogen to rats, as shown by several investigators, has been followed by a high incidence of mammary cancer. Using an inbred strain of albino rats, GESCHICKTER (1939) was able to induce a high incidence of mammary tumors by either injecting estrogens or implanting estrogen pellets. The latent period of tumor appearance was inversely proportional to the dose of estrogen given. Daily injections of large doses of estrogen shortened the induction period significantly. It was also noted that the induction period of mammary tumors varied with the type of estrogen used. Estradiol, which is biologically much more active than estrone, induced mammary tumors in 185 days, whereas an equal dose of estrone required twice as long to stimulate tumor development (GESCHICKTER AND BYRNES, 1942). Although GESCHICKTER did not stress the importance of chronological age in relation to the time of appearance of mammary cancer, his data nonetheless showed that the latent period of tumor development was inversely proportional to the age of the animal, tumors requiring 293 days for development in one-month-old rats, but only 90 days in those treated at 20 months of age. Administration of testosterone or progesterone simultaneously with estrogen, or immediately afterward, failed to inhibit mammary cancer development.

NOBLE *et al.* (1940) also reported a high incidence of mammary tumors, mostly adenocarcinomas, following subcutaneous implantation of small pellets of crystalline estrone in a hooded strain of rats only five to seven days old. The latent induction period was between 226 and 341 days. Unlike GESCHICKTER, NOBLE *et al.* found that the latent period of tumor induction was unaltered by increased dosage of estrogen.

Later, extended studies on estrogen-induced mammary tumors in rats showed that susceptibility to mammary tumor induction by

estrogen varied in different strains of inbred rats (DUNNING *et al.*, 1952). The effect of dietary fat and carbohydrate on estrogen-induced mammary cancer in rats previously had been studied by DUNNING *et al.* (1949). Dietary restriction to 22 calories per day caused death of the majority of the rats within 60 days. In the surviving rats, however, the caloric restriction did not reduce tumor incidence (which was 87%), but the latent period of tumor development was increased from 300 days to 400 days. A high-fat diet tended to accelerate the growth rate of the induced tumors. In these rats, hypertrophy and increased secretory activity of the mammary gland were observed. The importance of the tryptophan content of the diet in estrogen-induced mammary cancer in rats was also extensively investigated by DUNNING *et al.* (1950). The addition of 1.4% DL-tryptophan to a synthetic diet containing 25% tryptophan-free casein hydrolysate increased the number and percentage of diethylstilbestrol-induced mammary cancers and significantly reduced the average latent period of tumor development below that observed for rats in the control group which received an isocaloric 26% casein diet. These same investigators later showed that when a diet deficient in the essential amino acid, tryptophan, was fed to rats treated with diethylstilbestrol, the incidence of tumors was lower, but the survival time of the rats receiving the tryptophan-deficient diet was considerably reduced (DUNNING AND CURTIS, 1954).

Estrogen-induced mammary cancer metastasizes only occasionally. The hormone dependency of estrogen-induced mammary cancer in rats was first described by NOBLE AND COLLIP (1941). They found that surgical removal of implanted estrogen pellets from rats bearing estrogen-induced mammary cancer was followed by a rapid regression of all mammary tumors in four rats. When estrogen pellets were reimplanted into two of the four rats, the mammary tumors reappeared. These experiments were confirmed later when repeated under the direction of the same senior investigator (NOBLE AND CUTTS, 1958).

The induction of mammary cancer in rats by estrogen has been studied by only a few groups. The long latency of tumor development is apparently a great disadvantage to its usefulness as an experimental tool. Nonetheless, this intriguing question remains to be answered: How do estrogens induce neoplasia?

3. Pathophysiological changes in the pituitary gland after prolonged estrogen administration

It has been well established that a normal function of estrogens is to arouse and control various reproductive processes and that they are physiologically active in many parts of the body, in both male and female. Understanding the circumstances affecting these activities may help toward elucidating the mechanism of estrogen neoplasia.

The capacity of estrogen to induce or to stimulate the development of diverse neoplasia in various organs is amazing. The fact that the administration of an estrogen, under appropriate circumstances, will often be followed by development of a malignant tumor which otherwise would not have arisen, warrants the labeling of an estrogen as a carcinogen. Whether the estrogen is directly involved in the inductive process or primarily functions as a cofactor remains to be determined.

In considering experiments with mammary cancer induction by long continued estrogen administration, the first questions asked should be: 'What changes take place in the endocrine tissues after prolonged estrogen dosage? Are these changes related to the subsequent development of mammary cancer?'

Next to breast tissue, which undergoes extensive changes after repeated doses of estrogen, the most important endocrine organ showing dynamic changes in response to estrogen stimuli is the pituitary gland. The pathophysiological changes in this gland are both striking and consistent, the anterior pituitary gland enlarging, and the number of chromophobes markedly increasing. NELSON (1934) observed that estrone administration to castrated rats led to a great increase in the number of chromophobe cells. Most of the additional chromophobes were identified as degranulated basophiles, but to some extent the acidophiles were also degranulated. These findings were later confirmed by MARTINS (1936) and by WOLFE AND CHADWICK (1936). The observation that autologous pituitary grafts in the eyes of rats respond to estrogen stimuli in exactly the same way as does the intact pituitary led to the conclusion that estrogens act directly upon the anterior pituitary gland (MARTINS, 1936). Similarly, UOTILA (1940) found that sectioning of the pituitary stalk did not abolish the characteristic changes induced by estrogens.

The administration of large quantities of an estrogen induces hyperplasia and, ultimately, adenomas of the pituitary gland (Cramer and Horning, 1936; McEwen, Selye and Collip, 1936; Zondek, 1936). The point of interest in such pituitary, hyperplasia or adenoma is clearly that of function, i.e., do pituitary tumors secrete hormones, and if so, what is the hormonal effect on growth and neoplastic transformation in the mammary gland?

Several investigators (Zeckwer, 1944; Lacour, 1950; Bielschowsky, 1954) observed the formation of milk cysts in the mammary ducts of rats bearing estrogen-induced pituitary tumors. This led to the speculation that the mammary tumorigenesis was caused by stimulation of the prolactin-secreting cells. Hormone assays of estrogen-induced pituitary tumors in rats, as reported by Meyer and Clifton (1956), suggested that prolactin is the only hormone maintained at approximately normal concentration in the rapidly proliferating tissue. It should be noted, however, that such attempts to assay hormones from estrogen-induced pituitary tumors are fraught with difficulties, since there is no chemical method for purifying these hormones, and the accuracy of biological assay for quantitation of pituitary hormones is still in question. Until the purity of these tumors' hormones is chemically defined, any conclusion drawn from bioassay must therefore be accepted with reservations. Nonetheless, Furth and colleagues (1956) extensively studied the characteristics of estrogen-induced 'mammotrophic pituitary tumors', and observed that rats bearing mammotrophic pituitary tumor transplants manifested not only extensive hyperplasia and secretory activity in the mammary gland, but also increase in body weight and in the weights of the visceral organs. These observations led to the conclusion that 'mammotrophic pituitary tumors' are both mammotrophic and somatotrophic.

That pituitary prolactin is mammotrophic has been further substantiated by the work of Dao and Gawlak (1963). These investigators also demonstrated that subcutaneous grafting of single pituitary glands into hypophysectomized rats, or hypophysectomized and oophorectomized rats, induced profound alveolar lobular growth of the atrophic mammary glands. Even these experiments, however, fail to provide an answer to the question: Are the hormones released by the pituitary graft exclusively 'mammotrophic'? The presence of somatotrophic and adrenotrophic hormones in the pituitary graft has been reported (Hertz, 1959).

4. Pathophysiological changes in the pituitary gland in the estrogen-deficient rat

In rats receiving repeated estrogen treatment, the increased stimulation of mammary gland growth and secretory activity concurrently with hyperplasia or adenoma formation in the pituitary glands has led some investigators to conclude that the mammary gland changes are not the result of estrogen stimulation, but the effect of the mammotrophic hormones secreted by the hyperfunctioning pituitary gland. The action of estrogens on mammary glands is, in their opinion, mediated through the pituitary. Thus, they postulate that estrogen stimulates the pituitary gland to secrete mammotrophic hormone which, acting directly on the mammary gland, enhances its growth.

Although many experimental results support such a hypothesis, paradoxical observations have been made which show that mammary gland growth can be enhanced in rats deficient in estrogen as a result of gonadectomy. Pituitary basophil adenomas appearing in rats subsequent to gonadectomy have been reported (BIELSCHOWSKY AND HALL, 1951; HOUSSAY *et al.*, 1955; GRIESBACH AND PURVES, 1956). The pituitary tumors developing in rats chronically deficient in estrogen were classified as acidophil-chromophobe tumors. In these rats, it is most interesting to observe that whereas the uteri are atrophic, the mammary glands are sufficiently stimulated to contain milk. HOUSSAY *et al.* (1955) ascribed the mammary gland stimulation and the pituitary adenoma formation to the adrenal hyperplasia resulting from gonadectomy, since pituitary adenomas were formed only in animals with adrenal hyperplasia or adrenal adenomas. However, GRIESBACH AND PURVES (1960) found that none of the gonadectomized rats with pituitary adenoma showed any evidence of adrenocortical hyperplasia or adenoma function. In addition, they demonstrated that the incidence of pituitary adenoma is as high in gonadectomized-adrenalectomized rats as in rats that had only been gonadectomized. Although these investigators emphasized the regeneration of adrenocortical tissue after incomplete removal, they contend that no adrenal regrowth in the surviving 22 adrenalectomized-gonadectomized rats ever exceeded the size of the normal adrenal gland, and further, that such regrowth was always found on one side only. Apparently gonadectomy initially contributes to the development of pituitary hyperplasia or adenoma. These experiments clearly indicate that estrogenic hormones may not be

necessary for the stimulation of acidophils to secrete prolactin. The paradoxical experimental results concerning the role of estrogen in the pituitary's secretion of mammotrophic hormone cannot be explained readily. Further experiments to elucidate these results are urgently needed. Despite the work of GRIESBACH AND PURVES (1960), the role of adrenal hormones in the production of pituitary hyperfunction in gonadectomized rats must yet be clarified.

DAO AND GREINER (1961) reported that grafting of a pair of ovaries in castrated male rats induced marked enlargement and hyperplasia of the pituitary gland. Lactation was invariably observed in these rats. Histological examination revealed that the acidophilic and chromophobe cells were markedly increased in number and size. These authors reported adenoma formation in some of the rats. The adrenal gland in the castrated rats bearing functioning ovarian grafts were enlarged. These data suggest that gonadectomy contributes to the continuous stimulation of gonadotrophs by gonadal hormone deficiency and finally, pituitary hyperplasia and adenoma formation, the estrogen produced in the ovarian grafts perhaps enhancing the secretion and release of prolactin from these hyperfunctioning pituitary glands.

5. Relationship of the pituitary gland to estrogen effects on the mammary gland

Does estrogen exert a direct effect on the mammary gland in the absence of the pituitary gland? Unfortunately, since any experiment designed to elucidate this problem necessitates the use of hypophysectomized animals, interpretation of the data is complicated even at the start, and the question remains unanswered. Other complicating factors are uncertainties concerning the completeness of the hypophysectomy, the changes affecting the general well-being of the experimental animals, and the fundamental endocrine relationships. GOMEZ *et al.* (1937) showed that, in the presence of residual pituitary tissue amounting to as little as 2% of the total pituitary tissue excised, the mammary gland of hypophysectomized mice responded to estrogen stimuli the same as it did in intact mice. ASTWOOD *et al.* (1937) and NATHANSON *et al.* (1939) felt that the artificially induced inanition in hypophysectomized animals might also contribute to the negative responses to estrogen stimulation. Nevertheless, FREDRIKSON (1939) found that

administration of estradiol monobenzoate and progesterone caused as much alveolar ductal growth in the mammary gland in hypophysectomized rabbits as it did in intact rabbits. This observation confirmed the earlier work of ASDELL AND SEIDENSTEIN (1935), who used the same species, and LEONARD (1943) and SMITHEORS AND LEONARD (1943) demonstrated that species, age, and time of hypophysectomy may also affect the results. In view of the above-described conflicting results, it is not surprising that much confusion and disagreement has been aroused among the various groups of investigators.

Obviously, the mechanism by which estrogens affect the cells of the mammary gland remains to be elucidated. There has been no clear-cut experiment to show either that an estrogen acts on the mammary tissues directly, or that it stimulates the anterior pituitary gland to secrete mammotrophic hormones, thus acting on the mammary tissues indirectly. A third possibility which certainly cannot be dismissed is that of a synergistic relationship between the ovarian hormones and the pituitary mammotrophic hormones.

6. Conclusions

On the basis of the experimental results described so far, it seems evident that neither prolactin nor growth hormone from the anterior pituitary has any effect on mammary cancer induction in rats. On the other hand, prolonged administration of estrogen results in the development of mammary cancer in rats. Successful experimental studies of estrogen effects on mammary cancer induction in hypophysectomized rats, however, have not been reported.

Long-term administration of estrogen in rats produces changes, not only in the mammary gland, but also in the pituitary gland, those in the pituitary being both striking and consistent. In the case of completely hypophysectomized animals, however, the mammary gland changes have not been documented. Reports are controversial, some observers claiming that estrogen has no effect on the mammary glands in the absence of the anterior pituitary, and others stating that estrogen induces mammary gland growth in hypophysectomized animals. If a synergistic action involving both anterior pituitary and ovarian hormones is needed for

functional growth of the mammary gland, such interdependence would explain why the estrogenic hormone *per se* appears to be only indirectly concerned with mammary gland development and, hence, carcinogenesis.

A paradoxical observation is the development of pituitary tumors in rats chronically deficient in estrogen as a result of gonadectomy. The role of the adrenal gland in the induction of pituitary hyperfunction and mammary gland development remains to be clarified.

III. Effects of Polycyclic Hydrocarbons

1. Route of administration

a) Skin painting. The capacity of polycyclic hydrocarbons to induce mammary cancer was discovered accidentally, in experiments actually designed to study skin carcinogenesis in mice (MAISIN AND COOLEN, 1936). The induction of mammary cancer in rats by painting the skin with polycyclic aromatic hydrocarbons was first reported by ORR (1955). Rats of three different strains were painted daily with a solution of either 0.5 or 1.6% 7,12-dimethylbenz(a)-anthracene (7,12-DMBA)*. Mammary adenocarcinomas developed in 73% of the animals in both groups. The mean latent period of tumor development was 12.6 weeks in rats receiving the higher dose, but twice as long in those receiving the lower dose. Painting the skin with methylcholanthrene, however, failed to elicit any mammary tumors in 15 months.

The mechanism of remote carcinogenesis remains to be clarified. This problem will be discussed later in this chapter.

b) Subcutaneous and intramammillar injections. Injection of carcinogenic polycyclic aromatic hydrocarbons into the breast tissue leads to the formation of mammary tumors at the injection site. Injections of dibenz(a,h)anthracene (1:2:5:6-dibenzanthracene), benzo(a)-pyrene (3:4-benzpyrene), and 3-methylcholanthrene (3-MC) caused sarcomas, but only very occasionally, breast adenocarcinoma (DUNNING *et al.*, 1936, 1940).

* The old nomenclature of 3-methylcholanthrene is 20-methylcholanthrene and 7,12-dimethylbenz(a)anthracene is 9,10-dimethyl-1,2 benzanthracene.

In the present author's laboratory, injection of 3-methylcholanthrene into mammary tissue induced mammary tumors but they were predominantly sarcomas.* Attempts to induce mammary adenocarcinoma by a single injection of 0.2 ml of olive oil containing 10 mg of 3-MC into the nipple failed (Table I). These observations suggest that subcutaneously injected carcinogens concentrate locally at injection sites where sarcomas subsequently develop. It also appears that the locally injected hydrocarbons fail to enter the mammary epithelial cells.

Table I

Induction of mammary tumors in rats by intra-nipple or subcutaneous injection of 3MC

Groups	No. of rats	Rats with tumors	Histologic type
Intra-nipple injection (intramammillar)	18	10	Fibrosarcoma 8 Adenocarcinoma 2
Subcutaneous injection	20	17	Fibrosarcoma 15 Adenocarcinoma 2

c) Intravenous injection. Mammary tumor induction in rats by repeated intravenous injections of carcinogenic hydrocarbons was first performed by GEYER *et al.* (1951). Twelve weekly intravenous injections of 7,12-DMBA in Sprague-Dawley rats induced mammary cancer in 32% of the animals. It is interesting to note that of several carcinogens studied, including 3-MC, 7,12-DMBA was the only effective agent. Later, GEYER *et al.* (1953) showed that intravenous injection of an emulsion containing 0.22 mg of 7,12-DMBA three times for one week and an identical dose three weeks later increased the incidence of mammary tumors to 80%. Induction of mammary cancer in rats by intravenous injections of 7,12-DMBA in fine emulsions was also reported later by HUGGINS *et al.* (1961).

* DAO, 1961: unpublished data.

d) Oral administration. Oral administration of carcinogenic hydrocarbons can be accomplished either by direct stomach tube feeding (accidentally discovered during an experiment designed to induce gastric cancer [SHAY *et al.*, 1949]), or by feeding in the diet, which often takes considerably longer than the former method.

It was found that 2 mg of methylcholanthrene, introduced daily into the stomach in female Wistar rats weighing 60 to 80 g, induced mammary adenocarcinoma in 82% of the animals within an average of 195 days. Ten years later, a simple technique worked out by HUGGINS *et al.* (1959)—feeding a larger dose of 3-MC (10 mg in 1 ml of sesame oil) daily for an average of 60 days—induced mammary cancer in all rats so treated. In Sprague-Dawley rats, mammary cancer induction was contingent upon dosage and the timing of administration of the carcinogenic hydrocarbon. DAO AND SUNDERLAND (1959), reporting similar observations, showed that daily oral feeding of 3-MC six days a week for 20 days was adequate to induce mammary cancer in 100 per cent of the rats treated.

Although HUGGINS *et al.* (1961) reported successful mammary cancer induction with a single feeding of 100 mg of 3-MC, attempts to prepare 3-MC at a concentration of 100 mg/ml olive oil have not been successful in the present author's laboratory. The maximal dose of 3-MC used in this laboratory was 50 mg in 1 ml of sesame oil. Feeding a single dose of 30 or 50 mg of 3-MC evoked mammary cancer in 40 or 50% of rats, respectively, with an average latent period of 135 days.

An important simplification of the technique for inducing mammary cancer with a polycyclic aromatic hydrocarbon was made by HUGGINS and colleagues (1961), who discovered that a single feeding of the potent carcinogen 7,12-dimethylbenz(a)-anthracene (7,12-CMBA), evoked mammary cancer regularly and rapidly within 60 days. This observation was subsequently confirmed (DAO, 1962), and it was found that the optimal tumor-induction dose was 20 mg.

2. *Mammary tumors induced by carcinogenic hydrocarbons*

a) Histological characteristics. Mammary cancers induced by oral administration of polycyclic aromatic hydrocarbons are predominantly adenocarcinomas. Tumors of other histological types,

such as ear-duct tumors, sarcomas, and fibroadenomas, have also been observed (SHAY *et al.*, 1952; DAO *et al.*, 1960; HUGGINS *et al.*, 1961).

Carcinogen-induced mammary cancers grow progressively. When a mammary cancer grows larger than 2 cm in diameter, central necrosis almost invariably occurs. If the tumor is allowed to grow without interference, it can reach an enormous size, and the host usually succumbs from hemorrhage and infections caused by necrosis.

Mammary adenocarcinoma induced by carcinogenic hydrocarbons may metastasize, but only occasionally: lymph nodes, lungs, and liver may be involved with metastases (SHAY *et al.*, 1952; DAO, 1959). The low incidence of metastases of the induced tumor is an intriguing phenomenon worthy of intensive study.

b) Hormonal dependence. Since most of the established mammary cancers induced by either 3-MC or 7,12-DMBA are hormone-dependent, they undergo a considerable diminution in size after ovariectomy, combined ovariectomy and adrenalectomy, or hypophysectomy; the tumor regression is accompanied by atrophic changes in the tumor cells (HUGGINS *et al.*, 1959; DAO AND SUNDERLAND, 1959). The duration of tumor regression varies. In most rats, the tumors disappear or remain small for two months after either ovariectomy or hypophysectomy. At the end of this period, many tumors begin to grow again, but their presurgical growth potentials are generally reduced.

Many of the established mammary cancers also regress when androgenic hormones are administered. Injection of 1 or 2 mg dihydrotestosterone a day in rats with 3-MC-induced mammary cancer caused tumor regression in about 80% of the rats (HUGGINS *et al.*, 1959). In the present author's laboratory, similar results were obtained when testosterone or cortisone was injected into rats bearing 3-MC-induced mammary cancers*.

c) Biochemical characteristics of mammary tumors induced by 3-methylcholanthrene. Respiration, glycolysis, and levels of soluble pyridine nucleotide-linked dehydrogenases in 3-MC-induced mammary cancer were measured by REES AND HUGGINS (1960). In these

* DAO, 1959: unpublished.

experiments, 3-MC-induced mammary cancers were found to have high aerobic and anaerobic glycolysis, a biochemical phenomenon common to all malignant tumors (WARBURG, 1930). Among the soluble pyridine nucleotide-linked dehydrogenases, lactic and malic dehydrogenases had much higher activity than did other dehydrogenases, both in 3-MC-induced mammary cancer and in normal hyperplastic mammary glands.

In hormone-dependent mammary cancers, ovariectomy increased the activity of lactic and malic dehydrogenases. The glucose-6-phosphate dehydrogenase level in mammary cancer decreased after ovariectomy. Injection of 10 μg of estradiol-17β in ovariectomized rats bearing 3-MC-induced mammary cancer prevented regression of the mammary cancer, and restored the levels of lactic and malic dehydrogenases to those found in mammary cancer in intact female rats.

3. The role of steroid hormones in mammary tumor growth

a) Initiation. On the basis of their earlier observations, the present author and his associates postulate that mammary carcinogenesis by chemical carcinogens involves two or more separate components, with perhaps independent mechanisms in the total process of mammary cancer induction (DAO AND SUNDERLAND, 1959; DAO, 1962). In discussing the roles of hormones in chemical mammary carcinogenesis, it is imperative initially to define as exactly as possible the implications of such terms as *initiation, promotion,* and *maintenance.*

In chemical carcinogenesis, a carcinogen is, by definition, essential to initiate the neoplastic changes which occur in the affected cells. Nevertheless, there exist experimental data indicating that the presence of certain hormones may be a *sine qua non* in the induction of mammary cancer by the carcinogen. Under such circumstances, the exposed cells may not change except through joint action of the carcinogenic hydrocarbon and the hormone. In this sense, a particular hormone is a participant in the process of *initiation.*

The word *promotion* here means the effect of a hormone in accelerating the development of palpable tumors. *Maintenance* indicates that progressive tumor growth is sustained by one or more

hormones in such a way that removal of the hormonal influence will cause the regression of tumor growth.

It is well established that oral feeding of 3-MC or 7,12-DMBA induces mammary cancer regularly and rapidly in intact female rats, but fails to elicit mammary cancer in intact males, even when the dosage is markedly increased (Huggins *et al.*, 1959; Dao and Sunderland, 1959). In intact male rats treated with 3-MC, a few mammary cancers will develop when ovaries are grafted into the rats (Dao and Greiner, 1961). The mammary tumor incidence rises markedly if the male rats are castrated before the ovaries are implanted. Evidently testicular hormones profoundly inhibit mammary carcinogenesis by 3-MC.

It has also been demonstrated that castration of female rats prior to administration of a carcinogen reduces the incidence of mammary cancer, but does not actually prevent tumor induction (Huggins *et al.*, 1959; Dao and Sunderland, 1959). Later, Dao (1962) reported that if carcinogenic hydrocarbons are given to female rats 30 or more days after castration, mammary cancers are not induced. If estrogen or progesterone were administered concurrently with 3-MC to ovariectomized female rats, mammary cancer incidence equaled that of intact female rats (Huggins *et al.*, 1959).

These experiments indicate that a carcinogenic hydrocarbon, when administered alone (with no ovarian hormones) fails to elicit mammary cancer. This fact raised the question whether hormones may be of critical importance in initiating mammary gland carcinogenesis.

A unique study recently was performed in an attempt to elucidate the role of ovarian hormones in the initiation of mammary carcinogenesis induced by chemical carcinogens. Using male rats which are completely immune to cancer induction by chemical carcinogens, Dao (1962) demonstrated that administration of a carcinogenic hydrocarbon alone failed to inflict damage upon the mammary epithelial cells in the absence of ovarian hormones. Administration of a maximal carcinogenic dose of 3-MC to castrated male rats induced mammary cancer in only 5 to 10% of the rats, whereas the mammary tumor incidence rose to 53 to 65% if ovaries were grafted into such rats similarly treated with 3-MC. However, if the ovaries were grafted 25 days after the castrated male rats were fed 3-MC, the tumor incidence did not rise. Further, if castration

and ovarian grafting were done after the male rats were fed 3-MC, the tumor incidence was again unchanged. These experiments show explicitly that effective induction of mammary cancer requires the presence of ovarian hormones at the time of 3-MC administration (Table II). A debatable point is that initiation could have occurred anyway, the absence of ovarian hormones in the host causing the death of the affected cells; however, this explanation is devoid of supporting evidence.

Table II

Induction of mammary cancer in male rats (30 MG of 3-MCA given as single dose in all experimental groups)

Groups	No. rats	No. rats with tumors	Total no. of tumors	Appearance of palpable tumors (day)	
				Range	Mean
Castrated ♂	10	1 (10%)	1	126	
Castrated ♂ + Ov. graft → 3-MCA*	19	10 (53%)	11	40–106	102
Castrated ♂ + 3-MCA → Ov. grafts**	23	3 (13%)	3	135, 145, 148	
Intact ♂ + 3-MCA → castration + Ov. grafts***	27	1 (3%)	1	158	158

* Castration and ovarian graft done simultaneously in 35-day-old males, 3-MCA 25 days later.

** 3-MCA fed to 35-day-old male rats just being castrated; ovaries transplanted to these rats 25 days later.

*** 3-MCA fed to intact 35-day-old male rats; 25 days later, castration and ovarian graft done simultaneously.

(This table was published previously in Cancer Res. 22: 973–981 [1962]).

It is of interest that, in the experiments in which ovaries were grafted into castrated female rats subsequently fed 7,12-DMBA, no significant difference in mammary cancer incidence was observed in animals receiving ovarian grafts immediately after 7,12-DMBA feeding or 30 days later (Table III). This observation suggests that

so-called 'latent' tumor cells can survive through a prolonged period of ovarian hormone deficiency. Later contribution of ovarian hormones by the grafts only serves to promote the growth of the mammary cancer. It therefore follows that in the experiments with male rats early neoplastic initiation did not occur after the rats were fed carcinogenic hydrocarbons; otherwise mammary cancer certainly would have developed when ovaries were grafted into these rats.

These experiments present convincing evidence that the initiation of mammary carcinogenesis by means of polycyclic aromatic hydrocarbons requires the participation of ovarian hormones. Unfortunately, however, the exact mechanism by which the carcinogen and the ovarian hormones exert their joint action in the carcinogenic process is not yet clear.

b) Promotion and maintenance. Perhaps the most graphic demonstration of the role of steroid hormones in promoting mammary cancer growth is the phenomenal effect of pregnancy and pseudopregnancy on mammary carcinogenesis induced by polycyclic aromatic hydrocarbons. In a study of these factors (DAO AND SUNDERLAND, 1959), pregnancy markedly accelerated the growth rate of 3-MC-induced mammary cancers. In rats which became pregnant after having been fed 3-MC, the latent period of tumor development was shortened, and the number of mammary tumors developing in each rat was significantly increased. Immediately after parturition, the induced mammary cancers all regressed; however, they resumed their rapid growth if the tumor-bearing rats were mated and became pregnant again. Pseudopregnancy in rats receiving 3-MC had a similar effect. It was therefore suggested that progesterone was probably the principal active hormone responsible for the rapid induction of mammary cancer. HUGGINS *et al.* (1959) reported that progesterone accelerated the induction of mammary cancer; the most rapid development of breast cancer in rats fed 3-MC occurred in animals injected concurrently with large doses of this hormone. It must be pointed out, however, that in pregnancy the placental estrogenic and chorionic hormones, as well as progesterone, play important roles in the mammary cancer induction by 3-MC.

Steroid hormones no doubt play an important role in maintaining the growth of carcinogen-induced mammary cancer. As pointed

Table III

Effects of ovarian grafts on incidence of breast cancer in castrated female rats receiving a single dose of 20 mg DMBA

Groups	Rats fed DMBA 7 days after castration			Rats fed DMBA 15 days after castration			Rats fed DMBA 30 days after castration
	No. rats with tumors*	Total no. tumors	Appearance of palpable tumors (days)	No. rats with tumors*	Total no. tumors	Appearance of palpable tumors (days)	No. rats with tumors*
Control (castrated)	1/20 (5%)	1	95	0/20			0/20
Ov. graft 0 days after DMBA	3/7 (43%)	9	43, 47, 50	3/7 (43%)	4	43, 45, 59	0/7
Ov. graft 7 days after DMBA	2/6 (33%)**	4	43, 43	3/7 (43%)	3	34, 40, 72	0/7
Ov. graft 15 days after DMBA	3/7 (43%)	7	43, 43, 49	2/7 (29%)	2	75, 85	0/7
Ov. graft 30 days after DMBA	2/7 (29%)	2	92, 101	2/7 (29%)	2	84, 85	0/7

* No. rats with tumors / No. of rats.

** One rat died 1 week after feeding of DMBA.

(This table was published previously in Cancer Res. 22: 972–981 [1962]).

out earlier in this chapter, ovariectomy or combined ovariectomy and adrenalectomy cause rapid regression of induced mammary cancers. In ovariectomized rats with regressing mammary cancers, the growth activity of mammary cancer can be restored by administration of ovarian hormones (DAO AND GREINER, 1961; STERENTAL *et al.*, 1963). HUGGINS *et al.* (1959) also demonstrated that ovariectomy-induced regression of a carcinogen-induced mammary cancer could be nullified if an estrogenic hormone were administered immediately following castration. These observations have subsequently been confirmed by many investigators, e.g., KIM AND FURTH (1960).

c) Carcinogenicity and chemical structure. Numerous attempts to correlate the carcinogenicity of polycyclic aromatic hydrocarbons with the molecular structures have been based on molecular orbital calculations as advocated by PULLMAN AND PULLMAN (1955), and on the formation of charge-transfer complexes, as reported by SZENT-GYORGYI (1960). Serious difficulties were encountered, however, when correlation of carcinogenicity with structure was based primarily on the compounds' electronic structures.

YANG *et al.* (1961) observed that carcinogenicity of the polynuclear aromatic hydrocarbons is due not only to the electronic factors, but also to a steric factor. These investigators postulated that a polycyclic hydrocarbon cannot be carcinogenic unless it bears a steric resemblance to an active steroid hormone. Among polycyclic aromatic hydrocarbons of similar electronic properties, the closer the steric resemblance to a steroid, the higher the carcinogenicity. One possible corollary of this postulate is that the polycyclic hydrocarbons may act upon the same biological sites as do steroid hormones. Carcinogenesis induced by these hydrocarbons may be the result of interference with normal steroid functions.

To these factors, HUGGINS (1962) added that the thickness of the molecule must resemble that of the base pairs of the relevant nucleic acid. He suggested that steroid hormones are actually not carcinogens, since steroids are not planar, and their thickness far exceeds that of the base pair. In short, as postulated by HUGGINS, the mammary carcinogens must resemble the base pairs of the nucleic acid in geometrical configuration, and they must be able to form molecular complexes.

** One rat died 1 week after feeding of DMBA.
(This table was published previously in Cancer Res. 22: 972–981 [1962]).

4. The role of pituitary hormones in mammary tumor growth

a) Initiation. While the mechanism of the carcinogenic action of hypophyseal hormones in mammary cancer induction in rats is not clearly understood, it is now well established that the mammotrophic hormones, prolactin and growth hormone, are prerequisite to development of the mammary gland. Furthermore, a certain degree of mammary development apparently must be attained before mammary tumors can be induced.

Are mammotrophic hormones essential in order to initiate neoplastic changes in the mammary epithelial cells at the time of exposure to a potent chemical carcinogen? To design experiments calculated to answer this important question would be extremely difficult, if not impossible. First of all, the experiments must necessarily involve the use of animals which have been both hypophysectomized and ovariectomized, or even adrenalectomized, as well. Such animals are highly susceptible to infection, and are generally in poor health, even under forced feeding. Secondly, there is always uncertainty regarding the completeness of the adrenalectomy, as well as the hypophysectomy, such doubt naturally creating a problem in data interpretation. Finally, hypophysectomized animals are extremely sensitive to the toxic effects of carcinogenic hydrocarbons and even to the weaker toxicity of estrogens. As a result, the animals may die within so short a time that the carcinogenic process cannot be studied adequately.

Suitable pituitary grafts continue to secrete prolactin, despite severance from the hypothalamic connection (DESCLIN, 1950; EVERETT, 1954; MÜHLBOCK AND BOOT, 1959). Using such grafts as a source of prolactin, DAO (1962) demonstrated that pituitary homografts transplanted into breast tissue in castrated female or male rats subsequently fed maximal carcinogenic doses of 3-MC, failed to increase mammary cancer incidence. The pituitary grafts in these rats survived and functioned, as shown by the normal histology of the grafts and the normal development of the mammary gland, even though the incidence of mammary cancer remained unchanged. Similar results were obtained when pituitaries were transplanted into hypophysectomized or ovariectomized-hypophysectomized rats.

One of the most significant observations in these experiments was that prolactin released by the pituitary graft exerted a direct

action on mammary gland growth. The atrophic mammary gland on the side of the pituitary graft insertion became normal in appearance, with a well-developed ductal alveolar system, whereas the contralateral mammary gland remained atrophic (Dao, 1962; Dao and Gawlak, 1963). Additionally, the average number of mammary tumors developing in a mammary gland bearing a functioning pituitary graft was not significantly different from the number of tumors developing in an atrophic gland. Evidently mammary cancer induction is not necessarily dependent on the extent of mammary gland development. This dissociation of function and tumor formation refutes the assumption that a mammary gland must be 'stimulated' by hormones in order to undergo carcinogenesis. These experiments also demonstrate that prolactin has little, if any, effect on the mammary cancer induction by 3-MC in rats. The presence of prolactin without ovarian hormones apparently is irrelevant to the induction of mammary cancer by chemical carcinogens.

It should be noted that, in mice, continuous stimulation of the mammary gland by mammotrophic hormones may produce hyperplasia and tumorigenesis only when the 'mammary tumor agent' is present. Mammotrophic hormones alone have no inductive capacity (Bern and Nandi, 1961).

The current problem is to determine whether ovarian hormones can induce both mammary gland growth and mammary tumors in the absence of the pituitary gland. During the past year, the present author and his associates have made a concentrated effort to study the effect of estrogens and anterior pituitary hormones on the induction of mammary cancer in rats treated with 3-MC or 7,12-DMBA. The survival of hypophysectomized and ovariectomized rats which received these agents is shown in Table IV, the data clearly indicating that the immediate mortality of rats receiving both estrogen and 7,12-DMBA is so high that prolonged experiments cannot be carried out to elucidate the problem.

In the present author's laboratory, rats were fed a high-protein diet and were observed for 30 days prior to an experiment in which they were hypophysectomized at age 40–50 days. At age 70–75 days, these animals were ovariectomized or orchiectomized, with concurrent pituitary grafting and subcutaneous implantation of estrogen pellets, each weighing 6–7 mg and containing 300μg diethylstilbestrol (5% estrogen in cholesterol). Absorption of the

Table IV

Survival after a single feeding of carcinogenic hydrocarbons in hypophysectomized-ovariectomized rats*

Groups	No. rats	Survival (days)				
		15–30	30–60	60–90	90–120	120–150
Hypophysectomized-ovariectomized	10				3	7
Hypophysectomized + 30 mg 3-MCA	6			1	5	
Hypophysectomized + pituitary grafts + 30 mg 3-MCA	6				6	
Hypophysectomized-ovariectomized + pituitary grafts + 15 mg DMBA	20		7	4	4	5
Hypophysectomized-ovariectomized + 15 mg DMBA	8	5	2			1
Hypophysectomized-ovariectomized + Estrogen pellet + 15 mg DMBA	15	15				
Hypophysectomized-ovariectomized + Estrogen pellet + pituitary grafts + 15 mg DMBA	13	8	1	2	2	

* Carcinogenic Hydrocarbon was fed to rats after either pituitary grafting or insertion of estrogen pellet.

estrogen pellets began rapidly after implantation. Of the hypophysectomized-ovariectomized rats bearing estrogen pellets, 15 died within 30 days after the feeding of 7,12-DMBA. In these 15 rats, the mean uterine weight was 148.0 $\pm$ 10.4 mg, about 4 times as much as that in hypophysectomized-ovariectomized rats without estrogen pellets (33.3 $\pm$ 4.7 mg). The mammary glands of these rats (hypoph-

Fig. 1 Whole mount of mammary gland removed from ovariectomized-hypophysectomized rat. Note the thin and scanty ductal growth. $\times$ 12

Fig. 2 Whole mount of mammary gland removed from ovariectomized-hypophysectomized rat 15 days after implantation of estrogen pellet. Note the increased ductal growth. $\times$ 12

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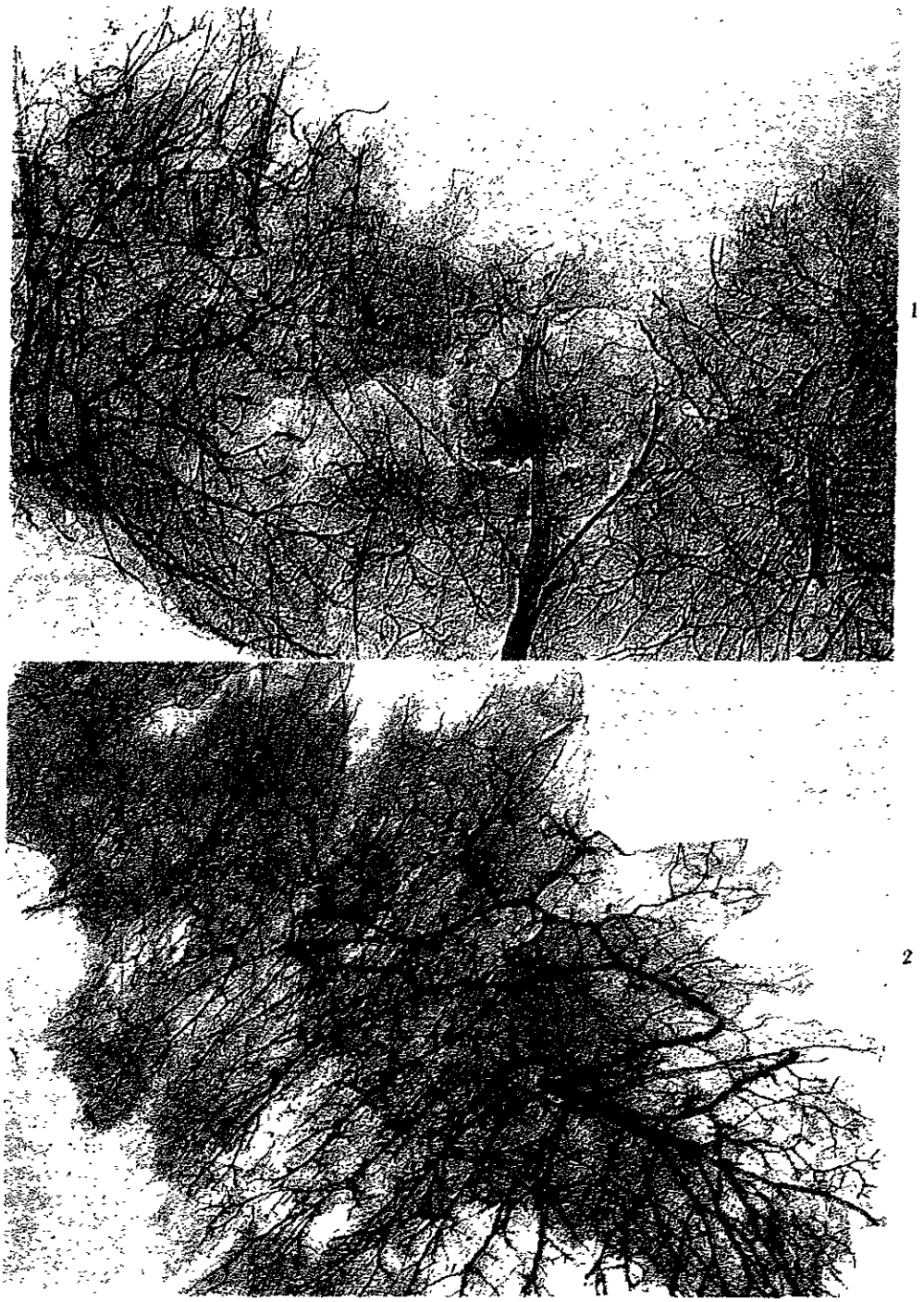

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Table V

Effect of a single feeding of DMBA on body, adrenal, and uterine weights in hypophysectomized-ovariectomized rats

Groups	No. rats*	Body weight (g)				Adrenal weight (mg)		Uterine weight (mg)	
		Initial		Final					
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Hypophysectomized-ovariectomized	9	103–212	108.8±5.4	98–179	148± 9.0	11.6–18.0	14.0±2.2	27.8– 40.6	33.3±4.7
Hypophysectomized-ovariectomized + 15 mg DMBA	8	105–109	107.6±1.1	82–112	98.8±12.1	11.4–15.0	12.6±1.4	23.4– 35.4	29.3± 5.2
Hypophysectomized-ovariectomized + estrogen pellet + 15 mg DMBA	10	105–122	110.9±5.4	77–102	89.7± 7.7	12.6–15.0	13.3±1.4	86.4–172.4	148±10.4
Hypophysectomized-ovariectomized + pituitary grafts + 15 mg DMBA	20	103–126	113.6±7.2	113–189	146.6±26.0	10.4–17.2	14.1±2.0	22.1– 43.4	34.4± 8.7
Hypophysectomized-ovariectomized + estrogen pellet + pituitary grafts + 15 mg DMBA	6	111–119	114.3±3.3	102–138	116.8±13.4	12.8–16.0	14.2±1.3	92.2–151.0	125.1±19.3

* The lesser number of rats in some groups as compared to the number of rats in the same experimental group as shown in Table 4 indicates rats died without autopsy (during weekends).

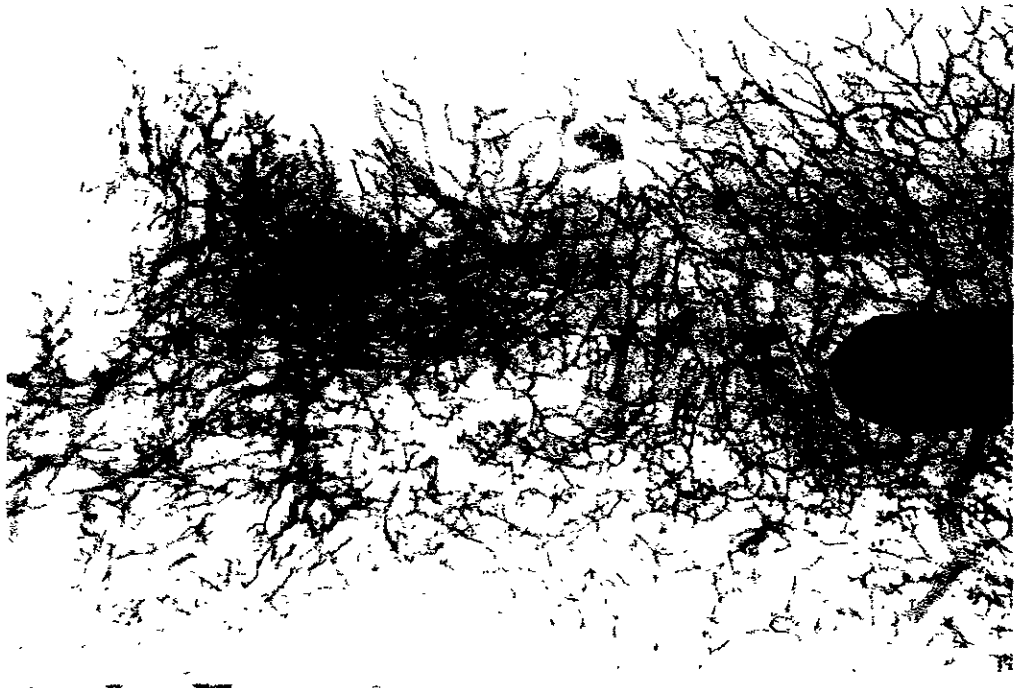
ysectomized-ovariectomized with estrogen pellets) surviving 15 days showed evidence of increased ductal growth, but there was no lobuloalveolar growth (Figs. 1 and 2). The question must be answered: Had the rats lived much longer, would the mammary glands have grown under the estrogen stimulation? All rats in this group lost weight and showed signs of inanition. The changes in body and organ weights are shown in Table V.

The toxic effect of a single feeding of 30 mg of 3-MC is clearly less pronounced than that of a single dose of 15 mg of 7,12-DMBA. (The dose of 7,12-DMBA was in fact reduced from 20 to 15 mg, in order to lessen the toxic effects.) None of the hypophysectomized rats fed a single dose of 3-MC, whether implanted with 2 pairs of pituitary grafts or not, had mammary cancer at the end of 4 months. Among the 21 hypophysectomized-ovariectomized rats receiving 2 pairs of pituitary grafts and a single dose of 15 mg of 7,12-DMBA, 6 lived 5 months, but none developed any mammary cancer. The other experimental groups provided no meaningful results, since all of the animals died too soon after 7,12-DMBA feeding. On the other hand, the mammary glands in the ovariectomized-hypophysectomized rats bearing pituitary grafts showed well-developed ductal alveolar growth.

In two hypophysectomized-ovariectomized rats, each bearing two pituitary grafts and one estrogen pellet and surviving 3 months, the mammary glands showed remarkable changes. In both rats, the right inguinal mammary glands in which the pituitary grafts were located showed extensive lobular alveolar growth and secretion, whereas their left inguinal mammary glands showed good ductal alveolar growth resembling that of an adult female rat's mammary gland (Figs. 3 and 4). Both rats were considered to be completely hypophysectomized, since autopsy revealed no evidence of remanent hypophyseal tissue. It appears that the changes in the left mammary glands of these rats were caused by the systemic effect of estrogen, since the prolactin secreted by the pituitary graft is insufficient to induce a systemic effect. The changes in the right mammary gland are attributable to the combined actions of estrogen and prolactin. These experimental results also suggest that prolonged stimulation of the mammary gland by estrogen can, to a certain extent, induce mammary gland growth.

Similar experiments carried out with male rats provided almost identical results to those obtained with female rats.

* The lesser number of rats in some groups as compared to the number of rats in the same experimental group as shown in Table 4 indicates rats died without autopsy (during weekends).



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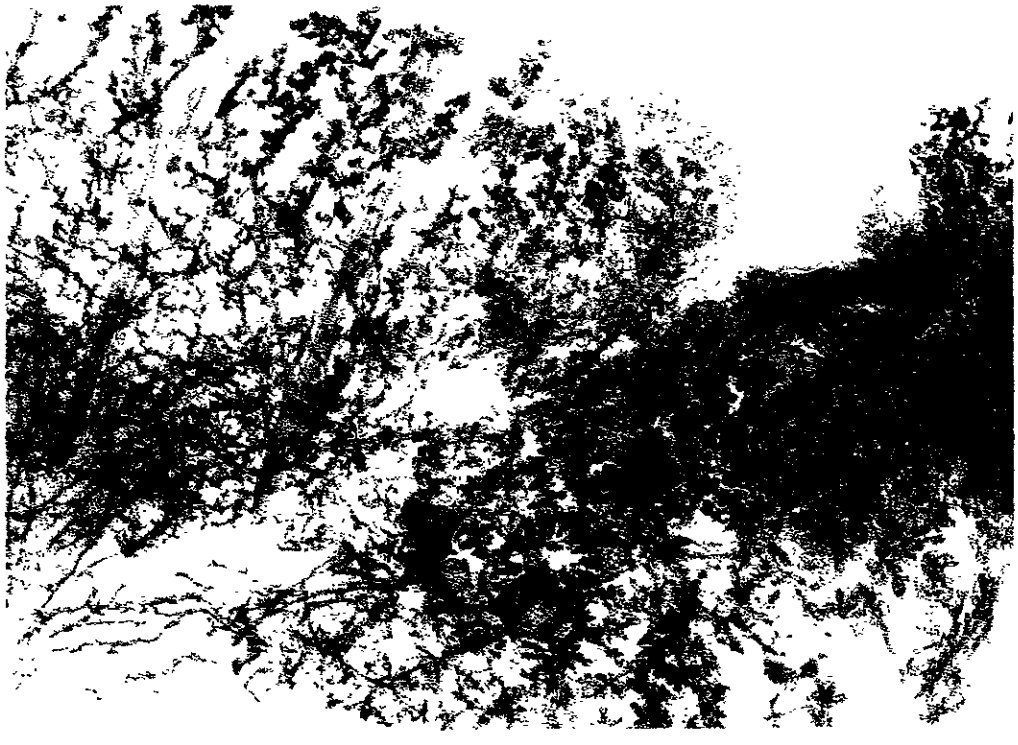

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b) Promotion and maintenance

In an established hormone-dependent mammary cancer, growth is necessarily maintained by the presence of hormones with mammotrophic activity. Thus hypophysectomy causes regression of hormone-dependent mammary cancers.

The question arises: How does the anterior pituitary maintain tumor growth—directly, by means of a mammotrophic hormone, or indirectly, by stimulating hormone production from the target organs? In other words, is it the somatomammotrophic hormones, the steroid hormones, or a combination of the two which is necessary for maintenance and progressive growth of the mammary tumor?

KIM AND FURTH (1960) reported that a 'subcarcinogenic' dose of 3-MC induced mammary tumors only in rats, the mammary glands of which were stimulated by mammotrophic hormone provided by tumor grafts. The mammary tumors regressing after ovariectomy and hypophysectomy were stimulated to regrowth when functional mammotrophic tumors were grafted into the rats. These experiments led the investigators to conclude that mammotrophic hormones from mammotrophic tumor grafts alone are adequate to sustain tumor growth.

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These data obviously do not provide conclusive evidence that a 'mammotrophic' hormone alone is adequate to maintain mammary tumor growth. Although many experiments show that the mammotrophic tumor secretes predominantly prolactin and somatotrophin (FURTH *et al.*, 1956), there are marked changes in the adrenal cortex in rats bearing mammotrophic tumor transplants. These changes cannot lightly be dismissed as adrenal hypertrophy due to some factor other than overproduction of adrenotrophic hormone.

Recently, COHEN AND KIM (1963) assayed the adrenotrophic activity of mammotrophic tumors in rats and mice, their results strongly suggesting that the so-called 'mammotrophic tumor'

Fig. 3 Whole mount of left abdomino-inguinal mammary gland removed from ovariectomized-hypophysectomized rat 90 days after implantation of estrogen pellet and grafting of 2 pituitaries in right inguinal region. Note the increased ductal-alveolar growth. ×12

Fig. 4 Whole mount of right abdomino-inguinal mammary gland removed from same ovariectomized-hypophysectomized rat. There are also 2 pituitary grafts on the same side. Note marked increase in lobul-alveolar growth. ×12

secretes not only mammosomatotrophic hormones, but also adrenotrophic hormone. The effects of prolactin on *corpora lutea* in the ovaries and on the secretion of progesterone in these rats must be taken into consideration. Repeated experiments (HUGGINS *et al.*, 1959; DAO AND SUNDERLAND, 1959) have shown that progesterone stimulates mammary tumor growth both in intact and castrated female rats. In the experiments of KIM *et al.* (1960) with hypophysectomized rats, could the overproduction of prolactin by the mammotrophic tumor perhaps have induced greater secretory activity of the *corpora lutea,* resulting in the production of progesterone? Also to be considered is the effect of overproduction of adrenotrophic hormones on the secretory activity of the adrenal glands.

A recent report by STERENTAL *et al.* (1963) indicates that the effect of ovarian hormones on 7,12-DMBA-induced mammary cancer is dependent upon the presence of the pituitary gland. Their data, although scanty and hence inconclusive, are interesting. Estrogen administration in hypophysectomized rats failed to stimulate mammary tumor growth, whereas a reactivation of tumor growth was observed in adrenalectomized-ovariectomized rats receiving similar treatment.

Similarly, KIM *et al.* (1963) concluded that estrogen in either small or large doses failed to stimulate the transplanted mammary adenocarcinoma in hypophysectomized rats. In contrast, in hypophysectomized rats bearing mammotrophic tumors (secreting mammotrophic hormones), the transplanted mammary adenocarcinoma grew progressively without estrogen. These experiments led to a similar conclusion that stimulation of mammary tumors in rats by small doses of estrogen is indirect and results from direct stimulation of pituitary mammotropes. These investigators attribute the fact that large doses of estrogen inhibit the growth of 'hormone-responsive' mammary tumors to the failure of stimulated mammotropes to release mammotrophic hormones. It should also be mentioned that a definite, perhaps non-specific, direct effect of estrogen on mammary tumor growth in hypophysectomized rats bearing mammary adenocarcinoma was observed in these experiments.